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PATENT
Docket No. 312762001530

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Ann MONOSOV and Xinyu Fu.

Serial No.: 09/023,232

Filing Date: 13 February 1998

For: NUDE MOUSE MODEL FOR HUMAN NEOPLASTIC DISEASE

Examiner: Ann Marie S. Wehbé

Group Art Unit: 1632

SECOND DECLARATION OF ROBERT M. HOFFMAN

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, ROBERT M. HOFFMAN, declare as follows:

1. I am a Professor in the Department of Surgery at the University of California San Diego Medical Center and am Chairman of the Board and Chief Executive Officer of AntiCancer, Inc., the assignee herein. I obtained my Ph.D. in biology from Harvard University in 1971 and have been practicing in the field of cancer research since that time. I have held academic positions at Harvard Medical School and at the Weizmann Institute and have published about 300 articles on subjects related to cancer and metastasis. I am on the editorial boards of *AntiCancer Research*, of *Clinical Cancer Research* and of *In Vitro Cellular and Developmental Biology*. My *curriculum vitae* was submitted along with my previous declaration and may be referred to, as needed, by the Office.

2. I have supervised research at AntiCancer, Inc., which employs the surgical orthotopic implantation of intact tumor tissue as claimed in the herein application. The research has resulted in the publication of numerous papers establishing that the model successfully mimics, in an immunocompromised rodent, the clinical course of tumor growth and metastasis. The results using this model are dramatically superior to those obtained by Kyriazis, Otto, Wang or McLemore.

3. In my previous declaration, I submitted a summary of results obtained in this work done under my supervision which confirmed that the views expressed in the foregoing paragraph 2 are indeed correct. These results were provided as Exhibits 2 and 3 to my previous declaration and copies of these documents are enclosed with this declaration as Exhibit A for the convenience of the Examiner. I note that an objection was made to the citation of the standard reference work of Holland, James E., *et al.*, editor, Cancer Medicine, 5th ed., B.C. Decker, Inc., Hamilton, Ontario, Canada (2000), as describing the known clinical course of various tumors on the basis that this document post-dates the application date herein by a dozen years. However, the description in this document merely updates a description providing substantially the same results in earlier editions of this same reference work.

4. In order to confirm that the metastatic patterns observed with the AntiCancer MetaMouse shown in the previously submitted exhibits is consistent with the clinical pattern of metastasis known at the time, I have attached copies of the appropriate pages from an earlier edition of Cancer Medicine published in 1973. The cover page and front-piece showing the date are attached as Exhibit B.

- Page 1 of Exhibit 2 previously submitted showed that when bladder cancer related to the RT-4 cell line was employed, metastases were found in liver, pancreas, diaphragm,

omentum, iliac lymph nodes, superficial inguinal lymph nodes, and gastric lymph nodes; when the bladder cancer related to RT-10 was employed, metastases were found in the liver, lung, pancreas, spleen, diaphragm, and lymph nodes. Exhibit C, which is pages 1670 and 1674 of the 1973 Cancer Medicine edition, states on page 1674 that bladder tumors that invade the bladder wall metastasize most commonly to the pelvic nodes and to distant locations including lung, liver and bone. As noted, metastases were indeed found in the liver and lung as well as the regional lymph nodes in our model.

- As noted on page 2 of Exhibit 2, previously submitted, for colon cancer of various types, metastases were always found in the liver and often in the lungs as well as in the mesenteric lymph nodes. Exhibit D, which is pages 1597 and 1600-1601 of Cancer Medicine, states on page 1601 that liver and lungs are overwhelmingly the most frequent sites of distant metastases.
- As set forth on page 3 of Exhibit 2, in our model, pancreatic cancer showed consistent metastases in the liver and intestinal organs and often in the lungs (MIA PaCa, BxPC-3 and Pan-12-JCK). As noted on page 1561 of Exhibit E (pp. 1559-1561 of Cancer Medicine), regional lymph nodes and liver are the most common sites and intestinal organs and lungs are frequently involved.
- As shown on page 4 of Exhibit 2, our breast cancer models showed metastases to the axillary lymph nodes, lung, liver and bone for MDA-MB-435 and to the lung for AC-2468. Exhibit F (which includes pp. 1772-1774 of Cancer Medicine) states on page 1773 that the leading sites of distant metastases are the lymph nodes, lungs, red bone marrow, liver and bone.

- Exhibit 3 attached to my previous Declaration showed that renal cell carcinoma metastasized to lung, lymph nodes and liver; Exhibit G which includes page 1656 of Cancer Research states that the lungs, bones and liver are the most frequent sites involved in metastases of kidney cancer.

5. In my previous Declaration, I also included two papers which indicated the ability of the invention model to mimic lung cancer and provided the results of studies using this model. To summarize, the Rashidi paper showed extensive metastases in the immediate area including the contralateral lung, and the mediastinal lymph nodes. In 40% of the model animals heart metastases occurred and brain metastases occurred in 30%. According to the Yang paper, widespread metastasis was found throughout the skeleton, as well as in the collateral lung. Exhibit H contains copies of the relevant pages of the 1973 edition of Cancer Medicine relating to the clinical progression of this condition. As shown on page 1484, extension of the tumor into the chest wall is very common and, as shown on page 1485, metastases to the supraclavicular lymph nodes, brain, abdominal organs and bone are also common. Brain metastases are often found at autopsy as set forth on page 1489.

6. Attached as Exhibit I, for the convenience of the Office, is a summary of the metastatic locations of various tumors as set forth in Exhibits C through H. In my professional judgment, the results that we have obtained and that were submitted in my previous Declaration demonstrate an unexpected superiority of the claimed model in immunocompromised rodents. It is, of course, understood that there is considerable individual variation in metastatic patterns. Not all patients with colon cancer, for example, exhibit metastasis to the lungs, but such metastases are found in many. The rodent model shows similar individual variability, but in general mimics the patterns found in patients.

7. I am familiar with the paper cited as a primary reference, Kyriazis, *Cancer Research* (1981) 41:3995-4000, with which the comparisons in Exhibit A (Exhibit 2) were made. It is evident from the paper itself that no particular advantage is seen by the authors with regard to the value of using intact tissue. Because I have done extensive work in this field, I understand that the use of intact tissue in a subcutaneous model is considerably more convenient than the use of cell suspensions, since the use of intact tissue does not require pretreatment of the excised tumor. Based on this added convenience, I believe that Kyriazis, *et al.,* utilized intact tissue, not in order to improve the results obtained in the model, but rather in order to save time and avoid inconvenience.

8. I was directly involved in the development of the models that are claimed in the present application, since this development took place at AntiCancer. At the time, the inventors were involved in studying the growth of tumors *in vitro*. One of the approaches employed was to provide a matrix for tumor growth, typically as a three-dimensional collagen gel. It was observed by the inventors that when tumor cells were cultured in this way, *i.e.*, preserving a controlled three-dimensional structure, their response to test protocols and compounds and the general behavior of the tumor growth was much more consistent with what is observed *in vivo* than was the case when tumors were simply cultured as cell suspensions. It was based on these observations that the inventors realized that the use of intact tissue, with its pre-imposed three-dimensional structure, would be preferable to the use of cell suspensions in any kind of tumor model. It was also appreciated by the inventors that an orthotopic model might offer an advantage. Because of the observations made on three-dimensional matrices, the present inventors came to the conclusion that the inconvenience of the complex techniques required to utilize intact tissue in an orthotopic model was worth tolerating, since they believed that the

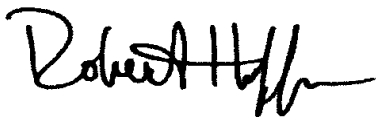
model might provide superior results as compared to other models known at the time. The results obtained when this inconvenience was tolerated and the present model was employed were indeed unexpectedly superior to what was contemplated. As shown in the results previously submitted, faithful reproduction of tumor progression in humans, including metastatic patterns was obtained.

9. It has been brought to my attention that the Office has taken the position that the work of Kyriazis in *Cancer Research*, cited above, observed metastases only in lymph nodes and lungs because other tissues were not examined. This is contradicted by the article itself which states that sections from various organs were fixed and examined histologically. As a worker in this field, I can testify that if histological examination had revealed metastases in other tissues, these would have been reported. Indeed, this is consistent with the observation made by the Office that in one case, metastasis was found in the diaphragm, and duly reported.

10. The value of the claimed tumor progression model is also evident from the high degree of commercial success that it has enjoyed. My company, AntiCancer, Inc., has received more than 100 service contracts from large pharmaceutical companies and other entities to employ the model as a contract service for testing of drugs and protocols. A significant portion of our company's income has been derived from such service contracts.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at San Diego, California, on 30 December 2003.



ROBERT M. HOFFMAN